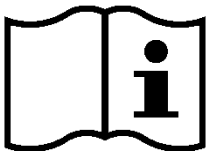


Useŕs Manual

Canine Anti-Thyroglobulin Antibody SRE

For the detection of antibodies against Thyroglobulin in serum and plasma samples of canine species

REF D3212-HR01

Σ 32

Nov 2021

Please use only the valid version of the package insert provided with the kit.

1. Table of Contents

2. Introduction

Antibodies against Thyroglobulin (TGAA) are an indication of auto-immune activity. This will normally induce thyroid dysfunction and a decrease in free T4. These antibodies account for the majority of canine hypothyroidism, circulating auto-antibodies that bind to thyroid hormones (THAA).
Higher values were found in ±5% of dogs between 1 and 6 years old. Highest rate off positivity was found in dogs with high TSH values (±12%). In several breeds a higher incidence was found; Golden retriever, Cocker spaniel, English setter, Old English sheepdog, Boxer, Shetland sheepdog and Beagle.

3. Intended use of the test kit

The anti-thyroglobulin kit is develop to detect antibodies against thyroglobulin and thyroglobulin complexes. To this end thyroglobulin and thyroglobulin complexes are bound to a solid phase.
After washing, the plates are incubated with the samples to be tested. The plates are washed after incubation to remove unbound material.
A HRPO conjugate is added to detect bound antibodies. After incubation and washing, the substrate is added and the optical density is measured at 620 nm.

4. Principle of the test kit

The test is based on the reaction of polyclonal antibodies reactive against thyroglobulin and thyroglobulin complexes.
The colour reaction in the wells is directly related to the concentration of antibodies in the serum/plasma sample.

5. Contents

- 4x 8 Microtiter strips coated with thyroglobulin and thyroglobulin complexes
- 2x Buffer (white bottle + green cap)
- 1x Negative control (ready to use) (brown cap)
- 1x Positive control (ready to use) (yellow cap)
- 1x Conjugate (black bottle + red cap)
- 1x Substrate A (white bottle + white cap)
- 1x Substrate B (black bottle + blue cap)
- 1x User's manual

Supplies needed (not included)

- Precision pipette 10-200µl (EVL)
- Pipette tips (EVL)
- ELISA plate reader (the results can be interpreted by eye, but for a more accurate and objective reading the use of the ELISA plate reader is strongly recommended)

6. Handling and storage of specimens

- The kit should be stored at 4°C.
- An open strip packet should be used within 28 days.
- Samples may be used fresh or may be kept frozen below -20°C before use.
- Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date.
- Avoid repeated freezing and thawing as this increases non-specific reactivity.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ±15 min. at room temperature (±21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solutions.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol **qualitative**

1. Before starting this test read **"preparations"**.
2. Open the packet of strips and take out the amount of wells needed from the test strip, 1 for each sample and 2 extra wells for the controls. Cover the remaining strips with a part of the provided seal and store them at 4°C and use them within 10 days.
3. Use the precision pipette 10-200µl and use a clean pipette tip **before** pipetting the buffer, standards, samples, conjugate and substrate.
4. Before testing make sure all reagents are at room temperature.

5. Wash the test strips with running tap water.
    - Fill all wells to the rim.
    - Empty the wells.
    - Repeat 5 times.
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

6. Add 240µl of buffer to all the wells, except control wells.
7. Add 100µl of the positive control to the first well.
8. Add 100µl of the negative control to the second well.
9. Add 10µl of sample (serum/plasma) to the remaining wells.
10. Mix the reagents gently (see **"preparations**").
11. Incubate for 40 minutes at room temperature (±21°C).

12. Wash the test strips with running tap water.
    - Fill all wells to the rim.
    - Empty the wells.
    - Repeat 5 times.
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

13. Add 100µl of conjugate to each well.
14. Incubate for 40 minutes at room temperature (±21°C).
15. Turn on the analyser (when available).

16. Wash the test strips with running tap water.
    - Fill all wells to the rim.
    - Empty the wells.
    - Repeat 5 times.
    - Turn the wells upside down and empty the wells by slapping the strips onto a tissue paper. Take care that none of the wells dry out before the next reagent is dispensed.

17. Add 60µl of Substrate A to each well.
18. Add 60µl of Substrate B to each well.
19. Mix the reagents gently (see **"preparations**").
20. Incubate for 10-15 minutes in the dark (e.g. cover the wells with a sheet of paper).

21. Read the absorbency values immediately (**within 10 min!**) at 620 nm on the analyser or by eye.
    Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

BV European Veterinary Laboratory
Postbus 198
3440 AD Woerden
The Netherlands

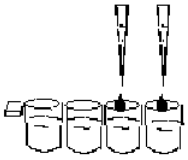
Tel: +31 (0)348-412549
Web: www.evlonline.org
@: info@evlonline.eu

9. Illustrated Test protocol

STEP: 5

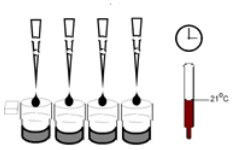
- Wash procedure
- Repeat 5 times

STEP: 6



- Add 240µl of buffer to all the wells, except control wells.

STEP: 7 t/m 11

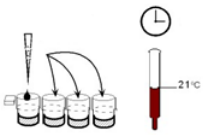


- Add 100µl of the positive control to the first well.
- Add 100µl of the negative control to the second well.
- Add 10µl of sample (serum/plasma) to the remaining wells.
- Mix the reagents gently (see “preparations”).
- Incubate for 40 minutes at ±21°C.

STEP: 12

- Wash procedure
- Repeat 5 times

STEP: 13 & 14

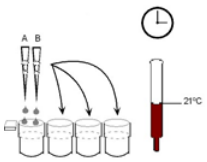


- Add 100µl of conjugate to each well.
- Incubate for 40 minutes at ±21°C.

STEP: 16

- Wash procedure
- Repeat 5 times

STEP: 17 t/m 21



- Add 60µl of substrate A to each well.
- Add 60µl of substrate B to each well.
- Mix the reagents gently (see “preparations”).
- Incubate for 10-15 minutes in the dark at ±21°C.

Read the absorbency values immediately (within 10 min!) at 620 nm on the analyser or by eye
Note: in case of using stop solution read the absorbency at 450 nm on the analyser.

10. Precautions

- Handle all biological material as through capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optimal cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

11. Interpretation of the test results

The analyser will give the results as positive, weakly positive or negative, but always double-check the outcome by observing the intensity of colour development.

- **Positive**
  - A sample is scored positive if the sample colour is dark blue, at least as blue as the positive control.

- **Weakly positive**
  - A sample is scored weakly positive if the sample colour is blue, with an intensity between that of negative and positive control.

- **Negative**
  - A sample is scored negative if the sample colour is equally blue or less blue than the negative control.

12. Symbols used with EVL ASSAYS

Symbol	Definition
	Consult instructions for use
	European Conformity
	In vitro diagnostic device
	For research use only
	Catalogue number
	Lot/ No. / Batch code
	Contains sufficient for <n> tests
	Storage Temperature
	Expiration Date
	Legal Manufacturer
Distributed by	Distributor
Content	Content
Volume/No.	Volume / No.

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